

and form extended surfaces in contact with the medium, the molecules of at least one third amphipatic substance associating with the extended surfaces, wherein

the at least one first and at least one second substance have different solubilities in the liquid medium,

the at least one first substance is less soluble in said liquid medium acting as the surface building substance,

the at least one second substance is more soluble in said liquid medium, forming less extended surfaces than the at least one first substance alone,

the extended surfaces formed by the first substance and the second substance are more extended than the surfaces formed by the first substance alone, and

the presence of said at least one second substance leads to an increase of the association of said at least one third substance with the extended surfaces formed by the at least one first substance and the at least one second substance.

59. The combination of claim 58 wherein the extended surfaces are in the form of membrane surfaces.

60. The combination of claim 58 wherein the extended surfaces, formed by the first, second and third substances carry a net electric charge, the molecules of the third substance associating with the extended surfaces, and the net charge density of the surfaces and the net charge of the molecules associating with the surfaces having the same sign.

61. The combination of claim 58 wherein the extended surfaces formed by the first, second and third substances are both negatively charged.

62. The combination of claim 58 wherein the extended surfaces formed by the first, second and third substances are both positively charged.

63. The combination of claim 58 wherein the second substance causes increased flexibility of extended surfaces formed by an at least one first substance being capable of self-aggregating, when being mixed with said at least one first substance.

64. The combination of claim 58 wherein the first substance and the second substance differ in solubility on the average at least 10-fold.

65. The combination of claim 58 wherein the first substance and the second substance differ in solubility on the average at least 100-fold.

66. The combination of claim 58 wherein the second substance increases the curvature of an extended surface formed by the first substance being capable of self-aggregating when being incorporated into the extended surface, the concentration of the second substance being below 99% of the saturation concentration, or of that concentration above which the extended surface could not be formed, whichever is higher.

67. The combination of claim 64 wherein the concentration of the second substance is at least 0.1 % of the relative concentration as defined in claim 8.

68. The combination of claim 64 wherein the concentration of the second substance is from 1 to 80 percent of the relative concentration as defined in claim 8.

69. The combination of claim 64 wherein the concentration of the second substance is at least 0.1 % of the relative concentration as defined in claim 8.

70. The combination of claim 64 wherein the concentration of the second substance is from 1 to 80 percent of the relative concentration as defined in claim 8.

71. The combination of claim 64 wherein the surfaces have an average curvature, yielding an average radius between 15 nm and 5000 nm.

72. The combination of claim 64 wherein the surfaces have an average curvature, yielding an average radius between 30 nm and 1000 nm.

73. The combination of claim 64 wherein the surfaces have an average curvature, yielding an average radius between 40 nm and 300 nm.

74. The combination of claim 64 wherein the surfaces have an average curvature, yielding an average radius between 50 nm and 150 nm.

75. The combination of claim 64 wherein the surface is supported by a solid.

76. The combination of claim 75 wherein the solid is a supporting surface of suitable curvature or size.

77. The combination of claim 60 wherein the relative concentration of surface-related charged components is between 5 and 100 rel. mole-% of the concentration of all surface-forming amphipats taken together.

78. The combination of claim 60 wherein the relative concentration of surface-related charged components is between 10 and 80 relative mole percent of the concentration of all surface-forming amphipats taken together.

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79. The combination of claim 60 wherein the relative concentration of surface-related charged components is between 20 and 60 relative mole percent of the concentration of all surface-forming amphipats taken together.

80. The combination of claim 60 wherein the average charge density on the surface is between  $0.05 \text{ Cb m}^{-2}$  and  $0.5 \text{ Cb m}^{-2}$ .

81. The combination of claim 60 wherein the average charge density on the surface is between  $0.075 \text{ Cb m}^{-2}$  and  $0.4 \text{ Cb m}^{-2}$ .

82. The combination of claim 60 wherein the average charge density on the surface is between  $0.1 \text{ Cb m}^{-2}$  and  $0.35 \text{ Cb m}^{-2}$ .

83. The combination of claim 60 wherein the concentration and the composition of electrolytes in which the first and the second substances are suspended, or from which the first and the second substances are adsorbed to a supporting surface, comprising mono or oligovalent ions, is chosen so as to maximise the positive effect of charge-charge interactions on the desired association and corresponds to ionic strength between  $I = 0.001$  and  $I = 1$ .

84. The combination of claim 60 wherein the concentration and the composition of electrolytes in which the first and the second substances are suspended, or from which the first and the second substances are adsorbed to a supporting surface, comprising mono or oligovalent ions, is chosen so as to maximise the positive effect of charge-charge interactions on the desired association and corresponds to ionic strength between  $I = 0.02$  and  $I = 0.5$ .

85. The combination of claim 60 wherein the concentration and the composition of electrolytes in which the first and the second substances are suspended, or from which the first and the second substances are adsorbed to a supporting surface, comprising mono or oligovalent

ions, is chosen so as to maximise the positive effect of charge-charge interactions on the desired association and corresponds to ionic strength between  $I = 0.1$  and  $I = 0.3$ .

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86. The combination according to claim 60 wherein the first substance is less soluble in the liquid medium, and/or being the surface-building and/or charge carrying amphipatic substance in the system, is a lipid, whereas the second substance is more soluble in the liquid medium, and/or causing increased surface curvature, flexibility or adaptability and/or being the charge carrying substance, is a surfactant, or is identical with the third substance.

87. The combination of claim 58 wherein the molecules are arranged in the form of minute fluid droplets suspended or dispersed in a liquid medium and surrounded by a coating of one or several layers of the first and second substances capable of self-aggregating and differing in solubility at least 10-fold in the liquid medium, such that the average diameter of homo-aggregates of the more soluble second substance or of hetero-aggregates of the first and second substances is smaller than the average diameter of homo-aggregates of the less soluble first substance.

88. The combination of claim 58 wherein the total content of all amphipats that form a surface is between 0.01 and 30 weight-% of the total dry mass of the aggregates.

89. The combination of claim 58 wherein the total content of all amphipats that form a surface is between 0.1 and 15 weight-% of the total dry mass of the aggregates.

90. The combination of claim 58 wherein the total content of all amphipats that form a surface is between 1 and 10 weight-% of the total dry mass of the aggregates.

91. The combination of claim 58 wherein the first substance is a biocompatible polar or non-polar surface-supporting lipid.

92. The combination of claim 91 wherein the first extended surfaces forming substance is capable of forming bilayers.

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93. The combination of claim 58 wherein the first extended surfaces forming substance is selected from the group comprising lipids, lipids from a biological source, corresponding synthetic lipids, and modifications of such lipids.

94. The combination of claim 93 wherein the first extended surfaces forming substance is selected from the group comprising glycerides, glycolipids, glycerophospholipids, isoprenoidlipids, sphingolipids, steroids, sterines or sterols, sulphur-containing lipids, a carbohydrate-containing lipids and half-protonated fluid fatty acids.

95. The combination according to claim 93 wherein the first extended surfaces forming substance is selected from the group comprising phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, phosphatidylserines, sphingomyelins, sphingophospholipids, glycosphingolipids, cerebroside, ceramidpolyhexosides, sulphatides, sphingoplasmalogenes, and gangliosides.

96. The combination according to claim 93 wherein the first extended surfaces forming substance is selected from the group comprising diacyl-, dialkenoyl- and dialkyl-lipids.

97. The combination according to claim 93 wherein the first extended surfaces-forming substance is selected from the group comprising lipids of the dioleoyl-, dilinoleyl-, dilinolenyl-, dilinolenoyl-, diarachidoyl-, dilauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl, or corresponding sphingosine derivative types.

98. The combination of claim 58 wherein the second substance is a surfactant.

99. The combination of claim 58 wherein the second substance is identical with the third substance.

100. The combination of claim 98 wherein the surfactant is selected from the group comprising nonionic, zwitterionic, anionic and cationic surfactants.

101. The combination of claim 98 wherein the surfactant is selected from the group, comprising long-chain fatty acids or long-chain fatty alcohols, alkyltrimethyl-ammonium salts, dialkyldimethyl-ammonium salts, trialkylmethyl-ammonium salts, alkylsulphate salts, monovalent salts of cholate, deoxycholates, glycocholates, glycodeoxycholates, taurodeoxycholates, taurocholates, acyl dimethyl-aminoxides, alkanoyl dimethyl-aminoxides, dodecyl dimethyl-aminoxide, alkyl-N-methylglucamides, alkanoyl-N-methylglucamides, N-alkyl-N,N-dimethylglycines, 3-(acyldimethylammonio)-alkanesulphonates, N-acyl-sulphobetaines, polyethylen-glycol-octylphenyl ethers, nonaethylen-glycol-octylphenyl ether, polyethylene-acyl ethers, nonaethylen-dodecyl ether, polyethyleneglycol-isoacyl ethers, octaethyleneglycol-isotridecyl ether, polyethylene-acyl ethers, octaethylenedodecyl ether, polyethyleneglycol-sorbitane-acyl esters, polyethylenglykol-20-monolaurate (Tween 20), polyethylenglykol-20-sorbitan-monooleate (Tween 80), polyhydroxyethylene-acyl ethers, polyhydroxyethylene-lauryl ethers, polyhydroxyethylene-myristoyl ethers, polyhydroxyethylene-cetylstearyl ethers, polyhydroxyethylene-oleoyl ethers, polyhydroxyethylen-4, or 6, or 8, or 10, or 12-lauryl ethers (Brij series), or in the corresponding esters, polyhydroxyethylen-8-stearate (Myrj 45), polyhydroxyethylen-laurate types, polyhydroxyethylen-oleate types, polyethoxylated castor oil 40 (Cremophor EL), sorbitane-monoalkylates (Arlacel or Span series), sorbitane-monolaurate (Arlacel 20, Span 20), acyl-N-methylglucamides, alkanoyl-N-methylglucamides, decanoyl-N-methylglucamide, dodecanoyl-N-methylglucamide, alkyl-sulphates, alkyl sulphate salts lauryl-sulphate, oleoyl-sulphate, sodium deoxycholate, sodium glycodeoxycholate, sodium oleate, sodium taurate, fatty acid salts, sodium elaidate, sodium linoleate, sodium laurate,

lysophospholipids, n-octadecylene-glycerophosphatidic acid, octadecylene-phosphorylglycerol, octadecylene-phosphorylserine, n-acyl-glycero-phosphatidic acids, lauryl glycerophosphatidic acids, oleoyl-glycerophosphatidic acid, n-acyl-phosphorylglycerol, lauryl-phosphorylglycerol, oleoyl-phosphorylglycerol, n-acyl-phosphorylserine, lauryl-phosphorylserine, oleoyl-phosphorylserine, n-tetradecyl-glycerophosphatidic acid, n-tetradecyl-phosphorylglycerol, n-tetradecyl-phosphorylserine, corresponding palmitoleoyl-, elaidoyl-, vaccenyl-lysophospholipids, corresponding short-chain phospholipids, and surface-active polypeptides.

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102. The combination of claim 86 wherein the surface formed by the at least one first substance and the at least one second substance contains charged membrane components in the relative concentration range between 1 to 80 mole percent.

103. The combination of claim 86 wherein the surface formed by the at least one first substance and the at least one second substance contains charged membrane components in the relative concentration range between 10 to 60 mole percent.

104. The combination of claim 86 wherein the surface formed by the at least one first substance and the at least one second substance contains charged membrane components in the relative concentration range between 30 to 50 mole percent.

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105. The combination of claim 58 wherein the surface-supporting at least one first substance is a phosphatidylcholine and/or a phosphatidylglycerol and the at least one second substance less capable of forming the extended surface is a lysophospholipid, a lysophosphatidic acid, methylphosphatidic acid, lysophosphatidylglycerol, lysophosphatidylcholine, a partially N-methylated lysophosphatidylethanolamine, a monovalent salt of cholate, deoxycholate, glycocholate, glycodeoxycholate, or a sufficiently polar sterol derivative, a laurate, myristate, palmitate, oleate, palmitoleate, elaidate or other fatty acid salt and/or a Tween-, a Myrj-, or a Brij-surfactant, or a Triton, a fatty acid sulphonate, -sulphobetaine, -N-glucamide or -sorbitane (Arlacel or Span) surfactant..



106. The combination of claim 87 wherein the average radius of the areas enclosed by said extended surfaces is between 15 nm and 5000 nm.

107. The combination of claim 87 wherein the average radius of the areas enclosed by said extended surfaces is between 30 nm and 1000 nm.

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108. The combination of claim 87 wherein the average radius of the areas enclosed by said extended surfaces is between 40 nm and 300 nm.

109. The combination of claim 87 wherein the average radius of the areas enclosed by said extended surfaces is between 50 nm and 150 nm.

110. The combination of claim 58 wherein the third substance associating with the extended surface comprises repeating subunits.

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111. The combination of claim 110 wherein the at least one third substance associating with the extended surface is a chain molecules, selected form the group comprising oligomers or polymers.

112. The combination of claim 111 wherein the chain molecules have an average molecular weight above 800 Daltons.

113. The combination of claim 111 wherein the chain molecules have an average molecular weight above 1000 Daltons.

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114. The combination of claim 53 wherein the chain molecules have an average molecular weight above 1500 Daltons.

115. The combination of claim 110 wherein the third substance is of biological origin.

116. The combination of claim 110 wherein the third substance is bioactive.

117. The combination of claim 58 wherein the third substance associates with the membrane-like extended surface by inserting itself in the interface and/or interfaces between the membrane and the liquid medium in contact with said membrane.

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118. The combination of claim 111 wherein the content of the chain molecules is between 0.001 and 50 relative percent compared to the mass of adsorbent surface, whereby the specific ratio value decreases with increasing molar mass of the chain molecules.

119. The combination of claim 111 wherein the content of the chain molecules is between 0.1 and 35 relative percent compared to the mass of adsorbent surface, whereby the specific ratio value decreases with increasing molar mass of said chain molecules.

120. The combination of claim 111 wherein the content of said chain molecules is between 0.5 and 25 relative percent compared to the mass of adsorbent surface, whereby the specific ratio value decreases with increasing molar mass of said chain molecules.

121. The combination of claim 111 wherein the content of the chain molecules is between 1 and 20 relative percent compared to the mass of adsorbent surface, whereby the specific ratio value decreases with increasing molar mass of the chain molecules.

122. The combination of claim 111 wherein the chain molecules are proteins, and at least a part of said molecules is associated with the surface, provided that such part has at least three segments or functional groups with a propensity to bind to said surface.

123. The combination of claim 111 wherein the chain molecules are polynucleotides.

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124. The combination of claim 123 wherein the polynucleotides are selected from the group comprising DNA and RNA, in the natural form or after chemical, biochemical, or genetic modification.

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Amk  
Q13*

125. The combination of claim 111 wherein the chain molecules are polysaccharides with at least partial propensity to interact with the surface either in the natural form or after chemical, biochemical, or genetic modification.

126. The combination of claim 58 wherein the third substance acts as an adrenocorticostaticum, a  $\beta$ -adrenolyticum, an androgen an antiandrogen, an antiparasiticum, an anabolicum, an anaestheticum, an analgesicum, an analepticum, an antiallergicum, an antiarrhythmicum, an antiarteroscleroticum, an antiasthmaticum, a bronchospasmolyticum, an antibioticum, an antidrepressivum, an antipsychoticum, an antidiabeticum, an antidot, an antiemeticum, an antiepilepticum, an antifibrinolyticum, an anticonvulsivum, an anticholinergicum, an enzyme, a coenzyme or corresponding inhibitor, an antihistaminicum, an antihypertonicum, a biological inhibitor of drug activity, an antihypotonicum, an anticoagulant, an antimycoticum, an antimyasthenicum, an agent against Morbus Parkinson or Morbus Alzheimer, an antiphlogisticum, an antipyreticum, an antirheumaticum, an antisepticum, a respiratory analepticum or a respiratory stimulant, a broncholyticum, a cardiotonicum, a chemotherapeuticum, a coronary dilatator, a cytostaticum, a diureticum, a ganglium-blocker, a glucocorticoid, an antiflow agent, a haemostaticum, a hypnoticum, an immunoglobuline or its fragment, an immunologically active substance, a bioactive carbohydrate, a bioactive carbohydrate derivative, a contraceptive, an anti-migraine agent, a mineralo-corticoid, a morphine-antagonist, a muscle relaxant, a narcoticum, a neurotherapeuticum, a neurolepticum, a neurotransmitter or its antagonist, a peptide, a peptide derivative, an opthalmicum, a sympaticomimeticum or a sympathicolyticum, a para-sympaticomimeticum or a para-sympathicolyticum, a protein, a proteine derivative, a psoriasis drug, a neurodermitis drug, a

mydriaticum, a psychostimulant, rhinologicum, a sleep-inducing agent or its antagonist, a sedating agent, a spasmolyticum, tuberculostaticum, urologicum, a vasoconstrictor or vasodilatator, a virustaticum, a wound-healing substance, or a combination thereof.

127. The combination of claim 58 wherein the third substance is a growth modulating substance.

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128. The combination of claim 58 wherein the third substance has immunomodulating properties, and is selected from the group comprising antibodies, cytokines, lymphokines, chemokines and correspondingly active parts of plants, bacteria, viruses, pathogens, immunogens, or parts or modifications thereof.

129. The combination of claim 58 wherein the third substance is a bio-catalyst.

130. The combination of claim 58 wherein the third substance is an enzyme, or a co-enzyme.

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131. The combination of claim 58 wherein the third substance is a recognition molecule, selected from the group comprising adherins, antibodies, catenins, selectins, chaperones, or parts thereof.

132. The combination of claim 58 wherein the third substance is a hormone.

133. The combination of claim 58 wherein the third substance is insulin.

134. The combination of claim 133 wherein the insulin is human recombinant or humanised insulin.

135. The combination of claim 133 wherein the content of insulin is between 1 and 500 I.U. insulin/mL.

136. The combination of claim 133 wherein the content of insulin is between 20 and 400 I.U. insulin/mL.

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137. The combination of claim 133 wherein the content of insulin is between 50 and 250 I.U. insulin/mL.

138. The combination of claim 58 wherein the third substance is interleukin suitable for the use in humans or animals.

139. The combination of claim 138 wherein the third substance is selected from the group comprising IL-2, IL-4, IL-8, IL-10, and IL-12.

140. The combination of claim 138 wherein the combination contains between 0.01 mg and 20 mg interleukin/mL.

141. The combination of claim 138 wherein the combination contains between 0.1 mg and 15 mg interleukin/mL.

142. The combination of claim 138 wherein the combination contains between 1 mg and 10 mg interleukin/mL.

143. The combination of claim 138 wherein the interferon is selected from the group comprising Interferon alpha, beta and gamma.

144. The combination of claim 138 wherein the composition contains up to 20 relative wt-% interferon.

145. The combination of claim 58 wherein the third substance is nerve growth factor (NGF).

146. The combination of claim 145 wherein the NFG is human recombinant NGF.

147. The combination of claim 145 wherein the combination contains up to 25 mg NGF/mL suspension.

148. The combination of claim 145 wherein the combination contains up to 25 relative weight percent NGF.

149. The combination of claim 145 wherein the combination contains between 0.1 and 15 relative weight percent NGF.

150. The combination of claim 145 wherein the combination contains between 1 and 10 relative weight percent NGF.

151. The combination of claim 145 wherein the third substance is immunoglobulin (Ig).

152. The combination of claim 151 wherein the Immunoglobulin (Ig) is used in the form of an intact antibody, part of it, or a biologically acceptable and active modification thereof.

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153. The combination of claim 151 wherein the combination contains up to 25 mg immunoglobulin(Ig)/mL suspension.

154. The combination of claim 151 wherein the combination contains up to 25 relative weight percent Ig relative to total lipid.

155. The combination of claim 151 wherein the combination contains between 0.1 and 15 relative weight percent Ig.

156. The combination of claim 151 wherein the combination contains between 1 and 10 relative w-% Ig.

157. A method of preparing a combination according to claim 58 in the form of a formulation of a biologically, cosmetically and/or pharmaceutically active agent, comprising:  
selecting the at least one first and the at least one second substance, forming extended surfaces, when combined in contact with said medium, such that said extended surfaces formed by the at least one first and the at least one second substance attract and associate with the at least one third substance, being said active agent, to a greater extent than the surfaces formed by the at least one first substance alone and are more extended than the surfaces formed by the at least one first substance alone;

generating said combination of surface-forming at least one first and at least one second substances by means of controlled mechanical fragmentation, in the presence of the at least one third substance in the form of said agent molecules, such that said agent molecules associate with said extended surface formed by controlled mechanical fragmentation.

158. The method of claim 157 wherein the means of controlled mechanical fragmentation are selected from the group comprising filtration, pressure change or mechanical homogenisation, shaking, stirring, and mixing.

159. The method of claim 157 wherein the combination of surface forming at least one first and at least one second substances is permitted to adsorb to suitable supporting solid surfaces, and then with the liquid medium by adding one substance after another or several at a time, whereby at least one of the later surface-forming steps is carried out in the presence of the agent that subsequently associates with the solid-supported surface.

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160. The method of claim 159 wherein the adsorbing surfaces or their precursors, whether suspended in a liquid medium or supported by a solid, are first prepared by steps which include sequential mixing of the surface forming molecules of the at least one first and at least one second substances, and the associating molecules of the at least one third substance are then added and permitted to associate with the said surfaces.

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161. A method for the preparation of a formulation for non-invasive application of active agents, wherein surfaces capable of associating with said agent molecules are formed from an at least one first substance being an amphiphilic substance, an at least one hydrophilic fluid, an at least one second substance being an edge active substance or surfactant, an at least one third substance being said active agent and, in case, other customary ingredients, which together form said formulation by separately mixing the at least one first substance being an amphiphilic substance, the at least one second substance being an edge-active substance or a surfactant, the at least one hydrophilic fluid and the at least one third substance being said active agent, the resulting mixtures then being combined to subsequently induce the formation of the entities which associate with the agent molecules.

162. The method of claim 161 wherein the active agent is selected from the group comprising anti-diabetic agents, growth factors, immunomodulators, enzymes, recognition molecules, adrenocorticostatica, and adrenolifica.



163. The method of claim 161 wherein the amphiphilic substances are either used as such, or dissolved in a physiologically compatible polar fluid, comprising water or water-miscible fluids, or in a solvation-mediating agent, together with a polar solution.

164. The method of claim 163 wherein the polar solution contains at least one edge-active substance or a surfactant.

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165. The method of claim 163 wherein the formation of said surfaces is induced by substance addition into a fluid phase, evaporation from a reverse phase, by injection or dialysis, with the aid of mechanical stress,.

166. The method of claim 163 wherein the formation of said surfaces is induced by filtration, the filtering material having pores sizes between 0.01  $\mu\text{m}$  and 0.8  $\mu\text{m}$ .

167. The method of claim 163 wherein the formation of said surfaces is induced by filtration, the filtering material having pores sizes between 0.02  $\mu\text{m}$  and 0.3  $\mu\text{m}$ .

168. The method of claim 163 wherein the formation of said surfaces is induced by filtration, the filtering material having pores sizes between 0.05  $\mu\text{m}$  and 0.15  $\mu\text{m}$ .

169. The method of claim 163 wherein several filters are used sequentially or in parallel.

170. The method of claim 163 herein said agents and carriers are made to associate, at least partly, after formation of the adsorbing surface.